PB86-204666

Reproductive Effects Assessment Group's Review of the Mutagenicity of Vinylidene Chloride

(U.S.) Environmental Protection Agency
Washington, DC

1986

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. REC
EPA/600/J-86/065		PB86-204666

4. TITLE AND SUBTITLE	5. REPORT DATE
THE REPRODUCTIVE EFFECTS ASSESSMENT GROUP'S REVIEW OF THE MUTAGENICITY OF VINYLIDENE CHLORIDE (Journal Version)	1986
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
David Jacobson-Kram	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
same as #12	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Office of Health & Environmental Assessment Office of Research and Development U.S. Environmental Protection Agency Washington, DC 20460	14. SPONSORING AGENCY CODE EPA/600/21

15. SUPPLEMENTARY NOTES

REF: Environmental Mutagenesis, 8:161-169, January 1986

16. ABSTRACT

A large number of studies indicate that vinylidene chloride is mutagenic to bacteria and that this activity is largely dependent on microsomal activation. Vinylidene chloride was reported to produce positive results for gene reversion and conversion in yeast, which was also dependent on metabolic activation, and was positive in tradescantia. In mammalian systems vinylidene chloride failed to induce gene mutations in V79 cells at two separate loci, failed to induce chromosomal aberrations in mouse bone marrow in vivo, and failed to induce dominant lethals in either mice or rats. Vinylidene chloride was found to bind to DNA of mice exposed through inhalation and may have caused unscheduled DNA synthesis in the kidneys of similarly exposed mice.

Analysis of the data relating to the potential of vinylidene chloride to behave as a human germ-cell mutagen indicates that, based on the criteria established in EPA's Proposed Guidelines for Mutagenicity Risk Assessment, the evidence at the present time is classified as limited. This designation indicates that there are insufficient data on either mutagenicity or interaction with germ cells to classify the evidence as either sufficient or suggestive of potential germ-cell mutagenicity. However, available data also do not permit the classification of vinylidene chloride as a non-germ-cell mutagen.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	10
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

EPA/600/J-86/065
JOURNAL ARTICLE

Environmental Mutagenesis 8:161–169 (1986)

The Reproductive Effects Assessment Group's Review of the Mutagenicity of Vinylidene Chloride

David Jacobson-Kram

Reproductive Effects Assessment Group, Office of Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.

A large number of studies indicate that vinylidene chloride is mutagenic to bacteria and that this activity is largely dependent on microsomal activation. Vinylidene chloride gave positive results for gene reversion and conversion in yeast that was also dependent on metabolic activation, and was positive in tradescantia. In mammalian systems, vinylidene chloride failed to induce gene mutations in V79 cells at two separate loci, failed to induce chromosomal aberrations in mouse bone marrow in vivo, and failed to induce dominant lethals in either mice or rats. Vinylidene chloride was found to alkylate DNA of mice exposed through inhalation and may have caused unscheduled DNA synthesis in kidneys of similarly exposed mice. The studies on the mutagenicity of vinylidene chloride are evaluated in this review.

Key words: vinylidene chloride, mutagenicity, gene mutations, chromosomal aberrations, sister chromatid exchange, DNA repair

INTRODUCTION

Vinylidene chloride ($CH_2=CCl_2$), also refered to as 1,1 dichloroethene, 1,1-DCE and 1,1 dichloroethylene is a highly reactive, flammable, clear colorless liquid. The vinylidene chloride monomer is used predominantly in the production of copolymers with vinyl chloride and acrylonitrile. Production levels of vinylidene chloride were estimated to be over 13 billion pounds in 1984 [Webber, 1985]. It is estimated that over one million pounds per year are released into the environment, resulting in human exposure from contaminated air and drinking water [Dilling, 1977]. Vinylidene chloride is metabolized in the liver, resulting in the generation of several possible reactive intermediates including an epoxide, with the primary organs of toxicity being the liver and kidney. Administration of vinylidene chloride to pregnant rodents has

Received July 31, 1985; revised and accepted September 19, 1985.

Address reprint requests to Dr. David Jacobson-Kram, Reproductive Effects Assessment Group (RD-689), U.S. Environmental Protection Agency, 401 M St., S.W., Washington, DC 20460.

been reported to have fetotoxic and weak teratogenic effects. In general, vinylidene chloride has given negative results in cancer bioassays in rats, mice, and hamsters.

MUTAGENICITY IN BACTERIA

Numerous studies have shown vinylidene chloride to be a bacterial mutagen (Table I). Many of these studies have included vinylidene chloride as a positive control when analyzing the mutagenicity of chemicals that are gases at or near room temperature. A variety of exposure conditions have been utilized in these studies including standard plate incorporation, liquid suspension, and host mediation. The most commonly used method, however, involves exposure of bacteria to a defined atmosphere of vinylidene chloride in an enclosed vessel.

Bartsch et al [1975] tested vinylidene chloride in the Ames assay by using strains TA100 and TA1530, both of which respond to base-pair mutagens. Tester cells were exposed to 0, 0.2%, 2%, and 20% vinylidene chloride in air (v/v) for 4 hours. The concentrations of vinylidene chloride in the incubation medium after 2 hours of exposure to 0.2%, 2%, or 20% were 3.3×10^{-4} M, 3.3×10^{-3} M, or 3.3×10^{-2} M, respectively. These concentrations did not increase further with incubations of up to 7 hours. Dose-related increases in the mutant frequencies were seen at 0.2% and 2% vinylidene chloride and were dependent on the presence of phenobarbital-induced mouse liver S9 in the medium. The maximum response was seen in TA100 with an approximate ten-fold increase over baseline. The response decreased at 20% in both bacterial strains; the authors suggested that this may have been due to an inactivation of microsomal enzymes by vinylidene chloride. S9 derived from mouse liver was more effective at activation than S9 from kidney or lung. S9 derived from phenobarbital-treated mice was more active than S9 derived from untreated animals, and S9 from mice was much more active than S9 from rats. The presence of N-acetyl-cysteine or N-acetyl-methionine in the medium decreased the mutagenic response by 80%. These latter compounds can act as free radical scavengers via their nucleophilic sulphur groups.

In a later publication, Bartsch et al [1979] examined the effects of various chemical pretreatments on the activity of rat liver-derived S9 mix. Pretreatment with phenobarbital and 3-methylcholanthrene resulted in increased activity (200% and 150% of untreated controls, respectively), whereas pretreatment with pregnenolone-16-alpha-carbonitrile, aminoacetonitrile, dibenamine, and disulfiram resulted in decreased activity. In this same study, S9 derived from four human liver samples was also shown to be capable of activating vinylidene chloride to mutagenic products. On the whole, human liver S9 was only one-fifth as active as mouse S9.

In a recent study, Oesch et al [1983] compared the relative activities of S9 derived from various species and organs at activating vinylidene chloride to mutagenic products. The target organisms were five Ames tester strains (TA1535, TA1537, TA98, TA100, and TA92) and *Escherichia coli* WP2 *uvrA* cells. Cultures were incubated at 375, 2250, 4500, 10,500, and 22,500 ppm for 4 h. Positive results were seen at all doses with mouse liver S9 mix. The following sequence of activating potencies were observed in TA100: mouse liver and Chinese hamster liver > rat liver > human liver > Chinese hamster kidney > male mouse kidney > rat kidney and female mouse kidney. The last two sources generated S9s that were almost without activity. The authors demonstrated a correlation between pretreatment of animals

TABLE I. Summary of Mutagenicity of Vinylidine Chloride (VDC): Bacteria

Reference	Test system	Dose	Results
Bartsch et al [1975]	Ames assay: strains TA1530 and TA100	0.2, 2, and 20% v/v in air for 4 hr	Positive in both strains.
Bartsch et al [1979]	Ames assay: strains TA1530 and TA100	2% in air for 4 hr	Positive in both strains.
Oesch et al [1983]	Ames assay: strains TA1535, TA1537, TA98, TA100, and TA92; reverse mutation in *E coli* WP2 *uvrA*	375, 2,250, 4,500, 10,500, and 22,500 ppm in air for 4 hr	Positive in all strains.
Jones and Hathway [1978]	Ames assay: strain TA1535	5% in air for 72 hr	Positive
Greim et al [1975]	Forward and reverse mutation in *E coli* K12; forward mutation to 5-methyltryptophan resistance and reverse mutation at the gal, arg, and nad loci	2.5 mM in medium for 2 hr	Negative at all loci in absence of S9. In the presence of S9, positive for arg, negative at other loci.
Cerna and Kypenova [1977]	Ames assay: strains TA1950, TA1951, TA1952, TA1535, TA1538, TA100, TA98; female ICR mice as host mediators	Spot test: 1%, 10%, and 100%	Spot test: positive at 100%, negative at lower doses.
		Host mediation: 1/2 LD_{50} and LD_{50}	Host mediation: positive but not dose related.
Baden et al [1976]	Ames assay: strains TA98 and TA100	3% for 8 hr in desiccator; 3% for 1 hr in medium; 10% for 1 hr in medium	Negative minus S9 in TA100. Positive minus S9 in TA98. Positive in both strains with rat and human S9.
Baden et al [1977]	Ames assay: strains TA100 and TA1535	3% in liquid incubation for 2 hr	Positive in both strains
Baden et al [1978]	Ames assay: strains TA100 and TA1535	3% in liquid incubation for 2 hr	Negative in both strains in absence of S9; positive in both strains with Aroclor-induced rat S9; positive in TA100 with human S9
Simmon et al [1977]	Ames assay: strain TA100	5% in air for 3, 6, 9, and 12 hr	Positive, time-related increases
Malaveille et al [1977]	Ames assay: strain TA100	2% in air for 2, 4, and 6 hr	Positive, time-related increases
Waskell [1978]	Ames assay: strain TA100	5% in air for 4 and 6 hr	Positive, time-related increases

with vinylidene chloride and the activity of the microsomal enzyme glutathione transferase. They suggested that the presence of the enzyme activity may be a contributing factor in species and sex differences noted for vinylidene chloride toxicity and carcinogenicity.

Another study that examined the effects of species, organ, and induction state on the activity of S9 mix was reported by Jones and Hathway [1978]. Ames strain TA1535 was exposed to 5% vinylidene chloride for 72 h. S9 mixes derived from mouse liver and kidney were more efficient at activation than were S9 mixes derived from rat liver and kidney. In general, pretreatment of both rats and mice with Aroclor 1254 increased the activity of S9 mixes. S9 derived from uninduced marmoset liver failed to generate mutagenic products as did liver S9 from an uninduced human being. A second human-derived liver S9 mix from an individual after long-term phenobarbital treatment was active at generating mutagenic metabolites.

Baden and coworkers [Baden et al, 1976, 1977, 1978] have used both liquid incubation and gaseous exposure of several Ames strains to analyze the mutagenic properties of a variety of volatile anesthetics. In all these studies vinylidene chloride was used as a positive control. For the liquid suspension assay, the concentration typically used was 3% for 1 or 2 h, and the target organisms were TA100 and TA1535. For gaseous exposure, TA98 and TA100 were incubated in an atmosphere of 3% vinylidene chloride for 8 h. Typical responses in the presence of Aroclor-induced rat S9 mix averaged four to five times background. These authors have also shown that pretreatment of rats with an enzyme inducer such as Aroclor greatly enhances the activity of liver-derived S9 mixes, and that S9 mix derived from human liver is also active at transforming vinylidene chloride to mutagenic products [Baden et al, 1976, 1978].

A number of studies, in addition to those cited above, indicate that vinylidene chloride is positive in the Ames assay as well as in other bacterial tests. Simmon et al [1977] showed a time-related increase in the number of TA100 revertants exposed to an atmosphere of 5% vinylidene chloride in air for 3, 6, 9, and 12 h. Little activity was observed in the absence of S9 mix.

In a study characterizing the stability of metabolic activation systems, Malaveille et al [1977] showed time-dependent increases in the numbers of TA100 revertants after incubation in an atmosphere of 2% vinylidene chloride in air for 2, 4, and 6 h. The largest increases in the number of revertants were up to ten times background. In agreement with other studies, S9 derived from phenobarbital-treated mice was more active than S9 from untreated animals.

Similarly, Waskell [1978] showed a time-dependent increase in the numbers of TA100 revertants after incubation in an atmosphere of 5% vinylidene chloride in air for 4 and 6 h. This increase was dependent on the presence of a rat liver S9 mix.

Greim et al [1975], using a liquid suspension protocol, examined vinylidene chloride-induced reverse mutation at three loci (gal, arg, nad) and forward mutation at one locus 5-methyl-tryptophan (MTR) in *E coli* K12. Cells were incubated for 2 hours in a medium containing 2.5 mM vinylidene chloride and tested for reversion to prototrophy (gal, arg, nad) or resistance to MTR. A significant increase in the mutant frequency was seen at the arg locus (2.3 times control) when cells were incubated in the presence of phenobarbital-pretreated mouse S9 mix.

In a study reported in abstract form only, Cerna and Kypenova [1977] reported that vinylidene chloride was positive in the bacterial spot test and the Ames assay

employing host-mediated activation. In the spot test, 0.05 ml of vinylidene chloride at 100%, 10%, and 1% was tested employing strains TA1950, TA1951, TA1952, TA1535, TA1538, TA100, and TA98. Positive results in strains sensitive to both base-pair and frameshift mutagens were seen only for 100% vinylidene chloride. In the host-mediated assay, female ICR mice were injected with vinylidene chloride at the LD_{50} and one-half of the LD_{50}, and mutagenicity was tested in strains TA1950, TA1951, and TA1952. Although positive results were noted, they were inversely related to dose.

There has been speculation as to the nature of the ultimate mutagen formed during metabolic activation of vinylidene chloride. Bartsch et al [1975] have suggested that an alkylating intermediate is formed, since mutations occur in *Salmonella typhimurium* strain TA1530, which is reported by Ames et al [1973] to be specifically reverted by monofunctional alkylating agents. Nevertheless, an alkylating compound was not identified in the trapping agent, 4-(4′-nitrobenzyl)-pyridine, when a gas that contained vinylidene chloride was passed through a mouse S9-activating system, even though this system provided activated metabolites that mutated *S typhimurium* strain TA100 [Barbin et al, 1978]. It has also been suggested that an unstable oxirane, possibly 1,1-dichloroethylene oxide, may be formed during metabolism and that this metabolite plays a significant role in the mutagenic activity of vinylidene chloride [Greim et al, 1975; Bonse et al, 1975; Bartsch et al, 1979]. Presently, however, there is little evidence identifying the exact metabolic intermediates that constitute the ultimate mutagenic form(s) of vinylidene chloride.

MUTAGENICITY IN YEAST AND PLANTS

Vinylidene chloride was tested for the induction of point mutations and mitotic gene conversion in the diploid strain (D7) of the yeast *Saccharomyces cerevisiae* [Bronzetti et al, 1981] (Table II). Cells were treated in a suspension containing 10,

TABLE II. Summary of Mutagenicity of Vinylidine Chloride (VDC): Yeast and Plants

Reference	Test system	Dose	Results
Bronzetti et al [1981]	Reverse mutation and gene conversion in *Saccharomyces;* activation either with mouse S10 mix or host mediated in male Swiss albino CD mice	10, 20, 30, 40, 50 mM for 2 hr in suspension; host mediation: 400 mg/kg acute or 23 × 100 mg/kg given 5 days/wk plus 200 mg/kg on day of assay; total dose 2,500 mg/kg; cells collected 4 hr after the last exposure	Suspension: dose-related increase in revertants and convertants in the presence of Aroclor 1254 pretreated mouse S10; host mediation: in acute study, positive for revertants and convertants for cells isolated from liver and kidney and negative for cells from lung; similar results in chronic study
Van't Hof and Schairer [1982]	Forward mutation: score change in flower color from blue to pink	1288 ppm for 6 hr; 22 ppm for 24 hr	Negative for 1,288 ppm; positive for 22 ppm

20, 30, 40, or 50 mM vinylidene chloride in the presence and absence of mouse liver S10 mix. In a second procedure employing host activation, yeast cells were injected in the retroorbital sinus of male Swiss albino mice (CD strain) and collected after 4 h from the liver, lungs, and kidneys.

In the suspension assay, a dose-related increase in revertants (Ilv^+) and convertants (Trp^+) was seen in the presence of S10 isolated from Aroclor 1254-treated mice. The increases in revertants (up to eight times control) and convertants (up to five times control) were noted at vinylidene chloride concentrations that permitted more than 50% cell survival.

In the host-mediated assay, mice were given an acute oral dose of 400 mg/kg vinylidene chloride, or were chronically treated (100 mg/kg, 5 days/wk with a total of 23 administrations, plus 200 mg/kg on the day of the assay; total dose was 2,500 mg/kg). In the acute protocol, there was a ten-fold increase in the number of convertants and a twelve-fold increase in the number of revertants isolated from the liver, a six-fold increase in convertants and a five-fold increase in revertants isolated from the kidney, whereas neither end point was increased in cells isolated from the lung. In chronically exposed mice, convertants were increased 13-fold and 6-fold in the liver and kidney, respectively, and did not increase in the lung, whereas revertants increased 18-fold and 30-fold in the liver and kidney, respectively, and again were negative in the lung. 4-methoxyphenol, which is often added to vinylidene chloride as an antioxidant, was negative at a concentration ten-fold higher than normally found in vinylidene chloride.

The results of testing vinylidene chloride in tradescantia were reported in a Gene-Tox publication that evaluated the database associated with testing in this plant [Van't Hof and Schairer, 1982] (Table II). The hybrid clone of tradescantia used in these studies (4430) is heterozygous at a flower-color locus. The dominant gene results in a blue flower, whereas inactivation of this locus allows the expression of the recessive allele resulting in a pink flower. Vinylidene chloride was reported to give negative results after exposure to 1,288 ppm for 6 h, whereas positive results ($P < .05$) were reported after exposure to 22 ppm for 24 h. Clearly, these results are difficult to reconcile. Unfortunately, the original reference from which these results were derived was not cited in the Gene-Tox report.

MUTAGENICITY IN CULTURED MAMMALIAN CELLS

Vinylidene chloride was tested for its ability to induce gene mutations at the hypoxanthine guanine phosphoribosyl transferase and sodium-potassium ATPase loci in V79 cells [Drevon and Kuroki, 1979] (Table III). Cultured cells were exposed to 2% and 10% vinylidene chloride in air for 5 h. No cytotoxicity and no increase in the mutant frequencies were noted for vinylidene chloride-exposed cells incubated in the presence of S15 from mouse liver or in the absence of microsomal enzymes. Cells exposed to vinylidene chloride in the presence of rat S15 showed 76% and 96% cytotoxicity at concentrations of 2% and 10%, respectively. Again, there were no increases in the frequencies of mutants. Both rats and mice had been pretreated with phenobarbital, and positive results were reported when vinyl chloride was similarly tested.

TABLE III. Summary of Mutagenicity of Vinylidene Chloride (VDC): Mammalian Cells

Reference	Test system	Dose	Results
Drevon and Kuroki [1979]	Forward gene mutation at the HGPRT locus and membrane sodium-potassium ATPase locus in V79 cells	2% or 10% in air for 5 hr	Negative for mutations at both loci both in the presence and absence of mouse and rat liver S15 mix
Cerna and Kypenova [1977]	Cytogenetic analysis in female ICR mouse bone marrow	Acute: 1/2 LD_{50}; multiple: 1/6 LD_{50} × 5, 1 dose/day, 5 days	Negative
Reitz et al [1980]	Binding of radiolabeled VDC to DNA of liver and kidney in CD-1 mice and Sprague-Dawley rats	50 ppm and 10 ppm in air for 6 hr	Binding: higher levels seen in kidney than liver, more binding in mice than rats
	UDS in vivo in liver and kidney of CD-1 mice		UDS: significant increase in kidneys of mice given 50 ppm
Anderson et al [1977]	Dominant lethality in male CD mice	10, 30, and 50 ppm, 6 hr/day, 5 days	Negative for No. of implants, No. of females with ⩾one early deaths/pregnancy, early deaths as a % of total implants.
Short et al [1977]	Dominant lethality in male CD rats	55 ppm, 6 hr/day, 5 days/wk, 11 wk	Negative for pre- and postimplantation loss.

MUTAGENICITY IN MAMMALIAN CELLS IN VIVO

The single report on the cytogenetic effects of vinylidene chloride was published in abstract form [Cerna and Kypenova, 1977] (Table III). Female ICR mice were given vinylidene chloride in a single dose (one-half of the LD_{50}) or in repeated applications (one-sixth of the LD_{50}, five intraperitoneal doses given at 1-day intervals). Animals were killed 6, 24, and 48 h following the last injection, and the bone marrow was analyzed for aberrations. No increase in chromosomal aberrations was found in any treatment group.

The capacity of vinylidene chloride to alkylate DNA and induce unscheduled DNA synthesis in vivo was studied by Reitz et al [1980] (Table III). These investigators exposed CD-1 mice and Sprague-Dawley rats to ^{14}C-labeled vinylidene chloride vapor for 6 h at 10 and 50 ppm (mouse only). DNA was then extracted from the kidneys and livers and analyzed for covalently bound radiolabel. The greatest level of binding was found in the kidneys of mice exposed to 50 ppm (30 alkylations/10^6 nucleotides) followed by the kidneys of mice exposed to 10 ppm (11 alkylations/10^6 nucleotides). Livers of exposed mice showed lower, but still detectable, levels of binding. Rats exposed to 10 ppm vinylidene chloride showed 2.0 and 0.87 alkylations/10^6 nucleotides for the kidney and liver, respectively. Radioactivity was eliminated from the kidney and liver of 50 ppm-exposed mice in a biphasic manner, with a half-life of 83 and 91 h, respectively. Rates of elimination were dose related—the lower doses being eliminated more rapidly.

Unscheduled DNA synthesis (UDS) was measured in the kidneys and livers of mice exposed for 6 hours to 10 and 50 ppm vinylidene chloride. Immediately after vinylidene chloride exposure, the animals were injected with ^{3}H-thymidine alone or with ^{3}H-thymidine plus hydroxyurea. This latter drug is given "to block most of the replicative DNA synthesis." The authors claimed a significant increase in UDS only in kidneys of mice exposed to 50 ppm. No effects were claimed for liver at 50 ppm or for either organ at 10 ppm. The validity of all these data is questionable for two reasons. First, the control values for normal DNA synthesis varied from 12.9 to 80.5 cpm/mg DNA in the liver of otherwise untreated mice and from 2.57 to 18.5 cpm/mg DNA in the livers of hydroxyurea-treated mice. Second, the effects of hydroxyurea on normal DNA synthesis and UDS could not be separated since this compound failed to suppress all scheduled DNA synthesis. Thus, in the one instance where the authors claimed a significant increase in UDS, the specific activity of DNA was lower than in the control.

The effect of vinylidene chloride on the induction of dominant lethals has been studied in both mice [Anderson et al, 1977] and rats [Short et al, 1977] (Table III). In the mouse study, 8- to 12-wk-old CD males were exposed to 10, 30, and 50 ppm in air 6 h/day for a total of 5 days. Matings began immediately after dosing and continued (one male with two virgin females) for a total of 8 weeks. Females were killed in midgestation after 14 to 16 days of pregnancy. Vinylidene chloride at 50 ppm reduced fertility in treated males up to 6 wk following treatment. In none of the treatment groups were there significant effects on the number of implants, the number of females with $\geqslant$one early death, the mean number of early deaths per pregnancy, or the early deaths as a percent of total implants. Vinyl chloride, which was also tested in this study, was also reported to be negative. The positive controls, cyclophosphamide (given intraperitoneally) and ethylmethanesulphonate (given per os), gave positive responses.

In the rat study, male CD rats were exposed to 55 ppm vinylidene chloride in air 6 hr/day, 5 days/wk, for 11 wk [Short et al, 1977]. During week 11 of exposure, each male was housed with two unexposed virgin females for at least seven successive evenings or until the male mated with two females. Females were killed on day 13 of gestation, and the number of corpora lutea and implants were determined. As in the mouse study, there was a significant decline in the number of pregnant females produced by exposed males. No effect was observed regarding the number of corpora lutea per dam, number of implants per dam, percent implants per corpora lutea, or percent viable embryos per total implants.

REFERENCES

Ames BN, Durston WE, Yamasaki E, Lee FD (1973): Carcinogens are mutagens: A simple test combining liver homogenates for activation and bacteria for detection. Proc Natl Acad Sci USA 70:2281–2285.

Anderson D, Hodge MCE, Purchase IFH (1977): Dominant lethal studies with the halogenated olefins vinyl chloride and vinylidene chloride in male CD-1 mice. Environ Health Perspect 21:71–78.

Baden JM, Brinkenhoff M, Wharton RS, Hitt BA, Simmon VF, Mazze RI (1976): Mutagenicity of volatile anesthetics: halothane. Anesthesiology 45(3):311–318.

Baden JM, Kelley M, Wharton RS, Hitt BA, Simmon VF, Mazze RI (1977): Mutagenicity of halogenated ether anesthetics. Anesthesiology 46:346–350.

Baden JM, Kelley M, Simmon VF, Rice SA, Mazze RI (1978): Fluroxene mutagenicity. Mutat Res 58:183–191.

Barbin A, Planche G, Croisy A, Malaveille C, Bartsch H (1978): Detection of electrophilic metabolites of halogenated olefins with 4-(4-nitrobenzyl) pyridine or with *Salmonella typhimurium*. Mutat Res 53:150.

Bartsch H, Malaveille C, Montesano R, Tomatis L (1975): Tissue mediated mutagenicity of vinylidene chloride and 2-chlorobutadiene in *Salmonella typhimurium*. Nature 225:641–643.

Bartsch H, Malaveille C, Barbin A, Planche G (1979): Mutagenic and alkylating metabolites of haloethylenes, chlorobutadienes, and dichlorobutenes produced by rodent or human liver tissues. Evidence for oxirane formation by P-450 linked microsomal oxygenase. Arch Toxicol 41:249–277.

Bonse G, Urban T, Reichert D, Henschler D (1975): Chemical reactivity, metabolic oxirane formation and biological reactivity of chlorinated ethylenes in the isolated perfused rat liver preparation. Biochem Pharmacol 24:1829–1834.

Bronzetti G, Bauer C, Corsi C, Leporini C, Nieri R, Del Carratore R (1981): Genetic activity of vinylidene chloride in yeast. Mutat Res 89:179–185.

Cerna M, Kypenova H (1977): Mutagenic activity of chloroethylenes analyzed by screening system tests. Mutat Res 46:214–215.

Dilling WL (1977): Interphase transfer processes. II. Evaporation rates of chloromethanes, ethanes, ethylenes, propanes and propylenes from dilute aqueous solutions: Comparisons with theoretical predictions. Environ Sci Technol 11:405–409.

Drevon C, Kuroki T (1979): Mutagenicity of vinyl chloride, vinylidene chloride and chloroprene in V79 Chinese-hamster cells. Mutat Res 67:173–182.

Greim H, Bonse G, Radwan Z, Reichert D, Henschler D (1975): Mutagenicity *in vitro* and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation. Biochem Pharmacol 24:2013–2017.

Jones BK, Hathway DE (1978): Tissue-mediated mutagenicity of VDC in *Salmonella typhimurium* TA-1535. Cancer Lett 5:1–6.

Malaveille C, Planche G, Bartsch H (1977): Factors for efficiency of the *Salmonella* microsome mutagenicity assay. Chem Biol Interact 17(2):129–136.

Oesch F, Protic-Sabljie M, Friedberg T, Klimisch HJ, Galatt HR (1983): Vinylidene chloride: Changes in drug metabolizing enzymes, mutagenicity and relation to its targets for carcinogenesis. Carcinogenesis 4:104–108.

Reitz RH, Watanabe PG, McKenna MJ, Quast JF, Gehring PJ (1980): Effects of vinylidene chloride on DNA synthesis and DNA repair in the rat and mouse: A comparative study with dimethylnitrosamine. Toxicol Appl Pharmacol 52:357–370.

Short RD, Winston JM, Minor JL, Hong CD, Seifter J, Lee CC (1977): Toxicity of vinylidene chloride in mice and rats and its alterations by various treatments. J Toxicol Environ Health 3:913–921.

Simmon VF, Kauhanen K, Tardiff RC (1977): Mutagenic activity of chemicals identified in drinking water. In Scott D, Bridges BA; Sobels FH, (eds): "Progress in Genetic Toxicology." The Netherlands: Elsevier, pp 249–258.

Van't Hof J, Scharier LA (1982): Tradescantia assay system for gaseous mutagens. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res 99(3):303–315.

Waskell L (1978): Study of the mutagenicity of anesthetics and their metabolites. Mutat Res 57:141–153.

Webber D (1985): Basic chemical production grew strongly in 1984. Chem Eng News, 63:12–14.